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The role of sexual selection in purging the genome of induced mutations in the bulb mite (*Rizoglyphus robini*)

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ABSTRACT

Background: Based on the assumption that males burdened with fewer deleterious mutations achieve higher reproductive success, the authors hypothesized that sexual selection can decrease the mutation load of populations.

Hypothesis: Sexual selection improves viability after induction of deleterious mutations.

Organism: The bulb mite, *Rhizoglyphus robini* (Acari: Acaridae).

Methods: Using 1.5 krad of gamma rays, the progeny (F1) of irradiated males, heterozygous for deleterious mutations, were divided into two treatments. In the sexual selection treatment (SS+), both pre-copulatory and post-copulatory sexual selection was allowed. In the non-sexual selection treatment, male–male competition and female choice were experimentally excluded. Viability and fecundity selection were permitted in both treatments. To control for non-genetic effects, we simultaneously ran two similar treatments with progeny of non-irradiated males. We subsequently assayed viability of offspring (F2) produced after each treatment.

Results: Irradiation significantly decreased viability. But the irradiation × treatment interaction was not significant. Thus, the hypothesis that sexual selection increases the rate of elimination of deleterious mutations over that caused by viability and fecundity selection was not supported in the present study.

Keywords: condition dependence, deleterious mutations, good genes, maintenance of sex.

INTRODUCTION

Sexual selection arises from competition over access to mates (Darwin, 1871) and/or their gametes (Parker, 1979). Sexual selection leads to evolution of traits (mostly in males) used either in intra-sexual competition or to increase male attractiveness to females (Darwin, 1871; Andersson, 1994). However, other traits, such as mate searching ability and general health, can also influence male reproductive success (Whitlock and Agrawal, 2009; Agrawal and Whitlock, 2010). Deleterious mutations are likely to decrease male reproductive competitiveness. Apart from detrimental effects on health that will affect traits such as mate searching efficiency, fighting ability or

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sperm production rate, mutations may also affect male sexual ornaments, rendering males less attractive to females. This is due to these traits often being condition-dependent, and condition is likely to be affected by mutations at many loci (Cotton *et al.*, 2004; Tomkins *et al.*, 2004). Indeed, the evolution of sexual ornaments may be explained by their information content, that is, revealing the mutation load of potential mates (Rowe and Houle, 1996; Houle and Kondrashov, 2002).

If reproductive competition does indeed significantly increase the efficiency of selection against deleterious mutations, it would have important evolutionary consequences. For example, sexual selection has been hypothesized to play a role in maintenance of sexual reproduction. Sexually reproducing populations are expected to pay a 50% cost in potential reproduction growth rate due to the production of males (Maynard Smith, 1978). If at least half of the total selection against deleterious mutations is due to sexual selection, the cost of sex can be fully compensated (Agrawal, 2001; Siller, 2001). Hadany and Beker (2007) showed that is still the case if the possibility of facultative parthenogenesis is taken into account. Furthermore, sexual selection can also prevent extinction of bottlenecked populations and help in the purging of inbreeding depression (Whitlock and Bourguet, 2000; Agrawal and Whitlock, 2010; Jarzebowska and Radwan, 2010).

However, experimental evidence to support the role of sexual selection in reducing mutation load is limited. It has been shown that sexually selected traits are more sensitive to radiation than the average trait (Moller and Mousseau, 2003). Whitlock and Bourguet (2000) found that mutations of large phenotypic effects, and decreasing female fecundity, also decrease male reproductive success in *Drosophila melanogaster*, which suggests that sexual selection would help eliminate these mutations. Sharp and Agrawal (2008) compared the strength of sexual and non-sexual selection on eight visible mutations in *D. melanogaster* and found that sexual selection was stronger than fecundity or viability selection for five of them. It has also been shown that sexual selection accelerates the elimination of the alcohol dehydrogenase null mutation in *D. melanogaster* (Hollis *et al.*, 2009). However, the studies discussed above investigated mutations of large effects that are unlikely to represent typical mutations segregating in natural populations. In contrast, Radwan *et al.* (2004) relaxed selection to allow accumulation of spontaneous mutations in the bulb mite (*Rizoglyphus robini*), but found no evidence that sexual selection prevents the resulting fitness decline. However, the effects of relatively few spontaneous mutations that have accumulated over a dozen or so generations might be difficult to detect.

In a more powerful design utilizing mutagenesis, Radwan (2004) showed that sexual selection in the bulb mite helps to recover fitness decline caused by induced mutations. However, non-sexual selection in this study was much relaxed, as fecundity selection acting on females was eliminated. It is therefore unclear if sexual selection significantly helps to reduce mutational load when non-sexual selection is acting simultaneously. Here, we use the bulb mite system to investigate the impact of sexual selection on purging the genome of induced mutations in the presence of other components of natural selection, including selection on female fecundity. For inducing mutations we utilize ionizing radiation, which causes a wide spectrum of fitness effects (Evans and DeMarini, 1999), and can therefore mimic the range of naturally occurring mutations.

Sexual selection in *Rhizoglyphus robini* (Acari: Acaridae) involves both pre-copulatory competition between males and post-copulatory sperm competition (Radwan, 1997; Radwan and Klimas, 2001). Males in this species are dimorphic. So-called 'fighters' possess a thickened third

pair of legs that are used in direct male conflict, whereas 'scramblers' have unmodified legs. Fighters achieve higher reproductive success than scramblers in mixed populations, which may be due to their higher genetic quality (Radwan and Klimas, 2001). Females are known to copulate with multiple mates, leading to intense sperm completion (Radwan, 1997). Females mated to several males produce more fecund daughters than females mated with only one male (Konior *et al.*, 2001), which seems to be due to a positive genetic correlation between male sperm competitiveness and female fecundity (Kozielska *et al.*, 2004). Although females are highly promiscuous, mate choice has also been documented – females avoid mating with older males, which are likely to have more deleterious mutations than younger ones (Prokop *et al.*, 2007).

Here, we compare the effectiveness of two treatments, differing in opportunity for sexual selection, in recovering viability after mutagenesis. To control for maternal effects associated with differences between treatments in conditions encountered by females, treatments without mutagenesis were run simultaneously. If sexual selection helps to reduce mutation load above the level achieved by non-sexual selection, we would expect the difference in viability between grand-offspring of irradiated and non-irradiated males to be more pronounced in the sexual selection treatment (i.e. there should be a significant interaction between sexual selection and irradiation). A significant effect of sexual selection with no significant interaction would imply the action of maternal effects.

MATERIALS AND METHODS

The mites used in the experiment came from a stock culture derived from a colony of about 200 individuals found on onions in a garden near Krakow, Poland in 1998. The stock culture had been kept in the laboratory as a large population (>1000 individuals, subdivided into periodically mixed sub-populations) for about 200 generations before the study was undertaken. The mites were maintained at 23–25°C, >90% humidity and fed powdered yeast. Base populations were kept in plastic containers (2.5 cm diameter, 2 cm high). Individually isolated mites and groups of males and females were kept in 0.8 cm diameter glass tubes (2 cm high) with plaster of Paris bases soaked with water, and were provided with food *ad libitum*.

At the beginning of the experiment, larvae were isolated in individual tubes and reared to adulthood to obtain virgin adults. After emergence of adults, half of the males were irradiated with 1.5 krad (15 Gy) of gamma radiation from cobalt-60. All males were irradiated in individual glass tubes at the same time. Two days after irradiation, males were mated to non-irradiated virgin females. To obtain control treatments, non-irradiated males were mated to virgin females. The mites remained paired in individual tubes for 5 days. Ten eggs were collected from each pair and reared separately to obtain the progeny used in the sexual and non-sexual selection treatments.

Induced mutations, embryonic viability, and fecundity of F1 females

To confirm that male irradiation had an effect on their progeny's fitness, we compared the embryonic viability of a random sample of 15 female progeny (each from a different family) of irradiated males with the embryonic viability of progeny of non-irradiated males. A sample of 30 eggs was collected from each female. Embryonic viability was calculated as the proportion of eggs that hatched.

Selection treatments

A single male and single female were collected from each family fathered by irradiated males (F1 generation) and assigned to one of the two treatments: with and without sexual selection (SS+ and SS– respectively). In the SS+ treatment a group of five females and five males was placed in the same glass tube, so that the males had to compete for access to females and their gametes, whereas in the SS– treatment each group of five females was housed with one male. Thus, the SS+ treatment allowed for both pre-copulatory and post-copulatory competition, while in the SS– treatment sexual competition was eliminated.

To control for possible maternal effects arising from differences between treatments in environments experienced by females, we conducted control treatments identical to the ones described above except that the mites originated from non-irradiated males.

The experiment was run in two blocks. Each treatment was conducted in 20 replicates within each block. The mites remained mated for 5 days. After that time males were discarded and females were placed in new glass tubes where they laid eggs used for further procedures. Females from each replicate oviposited in the same glass tube, which allowed for selection on female fecundity (i.e. the progeny of more fecund females were more likely to be sampled for fitness essays).

Embryonic viability assay

An embryonic viability assay was conducted on the F2 generation (i.e. on progeny of mites that underwent the experimental treatments). A random sample of 30 eggs from each replicate was taken and placed in a fresh vial. Over the 7 days that followed, hatched larvae were removed and on the seventh day the remaining eggs that failed to hatch were counted. Acarid mites do not lay unfertilized eggs (Heineman and Hughes, 1970), so the proportion of eggs that hatched could be used as a direct measure of embryonic viability.

Statistical analyses

All analyses were done in Statistica 7.1. To estimate the irradiation effect, we compared embryonic viability of progeny of irradiated and non-irradiated males using Student's *t*-tests. The main hypothesis of the interaction between treatment and irradiation was tested using two-way analysis of variance (ANOVA) with embryonic viability as the dependent variable, irradiation and treatment as fixed factors, and block as a random factor. Normality of error distribution was verified by examination of p-plots.

RESULTS

Embryonic viability in F1 was significantly lower in the progeny of irradiated males (mean ± S.D. = 0.53 ± 0.145) than in the progeny of males originated from the stock culture (0.92 ± 0.170; $t_{38} = 7.51$, $P < 0.0001$). This shows that mutations induced in fathers had significant heterozygous effects in their offspring.

In F2, mean embryonic viability in the irradiation treatments was 0.78 ± 0.16 for the SS+ treatment and 0.77 ± 0.17 for the SS– treatment, whereas in controls it was 0.94 ± 0.10 and 0.95 ± 0.11 for SS+ and SS– respectively (Fig. 1). The ANOVA showed no significant

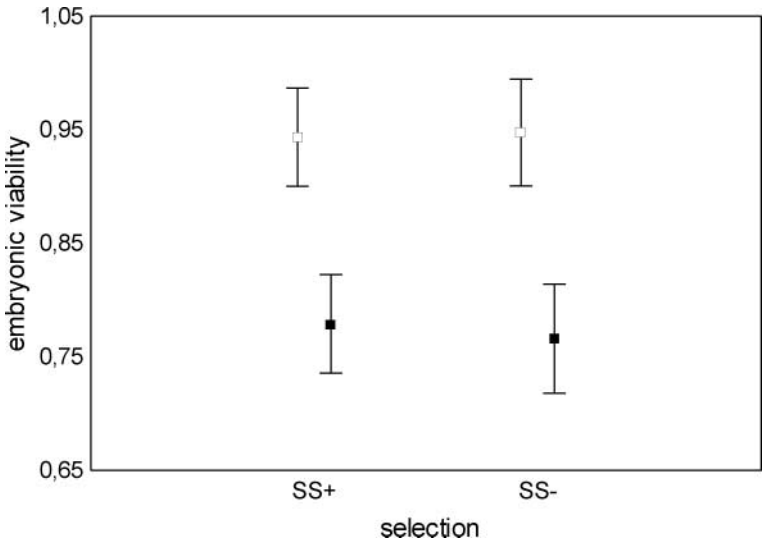

Fig. 1. Embryonic viability of offspring from treatments with and without sexual selection (SS+ and SS– respectively). ■ = treatments with induced mutations, □ = control treatments. Vertical bars denote 95% confidence intervals.

Table 1. Results of two-way ANOVA of embryonic viability

	d.f.	*F*	*P*	Effect size
Irradiation	1	54.97	<0.0001	0.2790
Sexual selection	1	0.006	0.937	0.0000
Irradiation × sexual selection	1	0.062	0.803	0.0004
Block	1	1.424	0.235	0.0099
Error	142	—	—	—

effect of block, but there was a significant effect of irradiation ($F_{1,143} = 56.565$, $P < 0.0001$). Importantly, neither the effect of treatment nor the treatment × irradiation interaction was significant (Table 1).

DISCUSSION

Sexual selection is expected to decrease the mutation load of populations if males' success in sexual competition decreases with the number of deleterious mutations (Agrawal, 2001; Siller, 2001; Houle and Kondrashov, 2002; Agrawal and Whitlock, 2010). In the present study, we found no evidence that sexual selection decreases the load of induced mutations more than fecundity and viability selection acting in the absence of inter-male reproductive competition. While irradiation of males significantly decreased embryonic viability of their grandchildren, confirming the effectiveness of the process of induction of deleterious mutations we applied, there was no significant interaction between irradiation and treatment. This

shows that treatments with and without sexual selection did not differ significantly in the effectiveness of purging induced mutations, although it is clear that our experimental design resulted in sexual selection in treatments where the males were able to compete for access to females. Radwan *et al.* (2004) reported a twofold increase in the variance in male reproductive success (a landmark of sexual selection) compared with random expectation using an identical design. Furthermore, the same design was used to demonstrate reproductive trait divergence under monogamy versus sexual selection (Tilszer *et al.*, 2006) and to show the role of sexual selection in the purging of deleterious mutations (Radwan, 2004) and of inbreeding depression (Jarzebowska and Radwan, 2010).

In contrast to the present results, in a study using the same species Radwan (2004) demonstrated that sexual selection is effective in removing induced mutations from populations. This was achieved via irradiation of bulb mite males whose subsequent sons were subjected to enforced monogamy or competition for access to females. Embryonic viability was higher among progeny produced in the sexual selection treatment than in the monogamy treatment, supporting the purifying role of sexual selection. The crucial difference between the two experiments is that in the current study fecundity selection of females was retained in both treatments, whereas in Radwan's (2004) study it was eliminated by enforcing the same contribution of each female to the next generation.

Thus, sexual competition does seem to select against deleterious mutations affecting viability, but its effect may only be detectable in the absence of fecundity selection acting on females. It therefore appears that sexual selection and fecundity selection do not act against deleterious mutations in an additive manner, which suggests that the former is unlikely to significantly compensate for the cost of producing males, as hypothesized by Agrawal (2001) and Siller (2001). Furthermore, Radwan *et al.* (2004) found no support for sexual selection preventing fitness deterioration when mutations were allowed to accumulate spontaneously under relaxed non-sexual selection [using the Middle Class Neighbourhood design of Shabalina *et al.* (1997)]. While that study had limited power, as the effects of spontaneously accumulating mutations are considerably weaker than those of induced mutations studied by Radwan (2004), the confidence intervals for the difference between SS+ and SS– treatments allowed Radwan *et al.* (2004) to conclude that sexual selection cannot fully compensate the twofold cost of producing males.

Our results contrast with those of Hollis *et al.* (2009), who showed that sexual selection increases the rate of decline in the frequency of the alcohol dehydrogenase null mutation (deleterious in the experimental context), despite the fact that their protocol allowed for female fecundity selection in both SS+ and SS– treatments. As the effect concerned only a single mutation of rather large effect on fitness, it is hard to say how general is the effect detected by Hollis *et al.* (2009). That sexual selection may be particularly effective against mutations of large effect is also suggested by Jarzebowska and Radwan (2010), who showed that sexual selection acting in small populations can be effective in purging the genome of inbreeding depression, but cannot prevent fixing mutations of small effect. More studies are clearly needed to assess the contribution of sexual selection to the process of purging populations of mutational load.

That we did not find a significant effect of treatment implies that exposing groups of females to either one or five males did not have an effect on the embryos in the eggs they laid. In contrast, Kozielska *et al.* (2004) found that sons (but not daughters) of polyandrous females have lower reproductive success than sons of monandrous females. This effect might possibly be mediated by a lower investment of polyandrous females in eggs (Kozielska

et al., 2004). However, while lower nutrient content of eggs is likely to affect growth and future performance of progeny, it is unlikely to affect survival at the embryonic stage.

In conclusion, although we found that irradiation of males influenced embryonic viability of their descendants, there was no evidence that sexual selection alleviates the detrimental effects of induced mutations. This contrasts with results of a previous study (Radwan, 2004) that investigated the effect of irradiation on the same fitness component. As discussed above, the crucial difference between the two studies is the simultaneous occurrence of female fecundity selection in the present study. Thus, it appears that although sexual selection does act against induced mutations (Radwan, 2004), its effectiveness depends on the simultaneous occurrence of other types of selection against deleterious mutations. Further empirical studies, using a broader range of mutational effects in realistic set-ups, are needed to understand the role of sexual selection in decreasing mutation load.

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